· 实验研究 ·

PEA3 activates CXCL12 transcription in MCF-7 breast cancer cells

CHEN Li, CHEN Bo-bin, LI Jun-jie, JIN Wei, and SHAO Zhi-min

【**Abstract**】 **Objective** To explore the activity of PEA3 (polyomavirus enhancer activator 3) on CXCL12 (Chemokine CXC motif ligand 12) transcription and to reveal the role of PEA3 involved in CXCL12-mediated metastasis and angiogenesis in breast cancer. **Methods** Methods such as cell transfection, ChIP assay (chromatin immunoprecipitation), and siRNA (small interfering RNA) were applied to demonstrate and confirm the interaction between PEA3 and CXCL12. **Results** Over-expression of PEA3 could increase the CXCL12 mRNA level and the CXCL12 promoter activity in human MCF-7 breast cancer cells. ChIP assay demonstrated that PEA3 could bind to the CXCL12 promoter in the cells transfected with PEA3 expression vector. PEA3 siRNA decreased CXCL12 promoter activity and the binding of PEA3 to the CXCL12 promoter in MCF-7 cells. **Conclusions** PEA3 could activate CXCL12 promoter transcription. It may be a potential mechanism of tumor angiogenesis and metastasis regarding of PEA3 and CXCL12.

【**Key words**】 polyomavirus enhancer activator 3 (PEA3); chemokine CXC motif ligand 12 (CXCL12) promoter; transcription regulation

Chemokine CXC motif ligand 12 (CXCL12), which is designated as stromal derived factor-1 (SDF-1), is a chemokine that binds to two receptors, CXCR4 and CXCR7[1-2]. It has been proposed that CXCL12 has various functions from developmental physiology to cancer biology, especially in some tumor growth and metastasis. CXCL12 is a chemoattractant for hematopoietic cells, endothelial cells and stem cells, involved in some developmental activities including developing nervous, cardiovascular and hematopoietic systems[3-4]. CXCL12 can trigger the

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phosphatidylinositol-3-kinase activating protein kinase AKT. AKT activation contributes to inhibition of apoptosis and to an increase in malignant cell proliferation, which is crucial for the development of breast cancer[5]. CXCL12 stimulates breast cancer cell lines motility and enhances their adherent ability to extracellular matrix (ECM) components[6]. It is reported that CXCL12-CXCR4 axis plays a pivotal role in the organ-specific metastasis of breast cancer. Metastatic breast cancer cells expressing the CXCR4 receptor have the tendency to attack the lungs, livers, and bone marrows, producing high levels of CXCL12[7]. Further, clinical studies demonstrated that the CXCL12 level in blood plasma in patients has a positive correlation with the risk of metastasis and poor prognosis[8].

Consequently, understanding the exact mechanism of CXCL12 regulation has a promising clinical value. Down-regulation of CXCL12 results in decreased migratory and invasive potential of breast cancer cells.

The CXCL12 expression may be regulated by epigenetic mechanism in breast cancer, including methylation of CpG islands of its promoter sequence[9-11]. Tamoxifen epigenetically activates the CXCL12 expression in breast cancer cells and makes these cells less susceptible to attraction by exogenous CXCL12 to metastasis sites[12]. Some transcription factors can also influence CXCL12 transcription. c-myb plays an important role in inducing the CXCL12 promoter activity by directly binding to the CXCL12 promoter[13]. The inhibition of CXCL12 signaling may provide potential targets for antiangiogenic therapy in several malignancies especially in breast cancer.

PEA3 is also a transcription factor, and so far there are few reports as to whether PEA3 can activate CXCL12 transcription. The purpose of this study was to explore the activity of PEA3 on CXCL12 transcription and to excavate the potential role of PEA3 in CXCL12-mediated metastasis and angiogenesis of breast cancer.

1 MATERIALS AND METHODS

1.1 Cell lines, culture, plasmids and transfection

Human breast cancer cell lines, MCF-7, which was purchased from ATCC, were maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin at 37 ℃ in a

humidified atmosphere of 5% CO_2 and 95% air. Cells were checked routinely and found to be free of contamination by Mycoplasma or fungi.

CXCL12 promoter/luciferase gene construct pCMVLUC-SDF1010 (−1010 to +122) and its negative control plasmid pDPROMLUC were kindly provided by Dr Antonio Caruz (Immunogenetics Unit, Faculty of Sciences, University of Jaen, Campus Las Lagunillas SN, Jaen, Spain). PEA3 expression vector was kindly provided by Dr Hassell (Department of Biology, McMaster University of Canada). Transfections were conducted using Lipo-fectamine method. Briefly, for transient transfection, cells were seeded in six-well plates at a density of 4×10^5 cells/well. The following day, cells were transfected with 4 mg of PEA3 expression vector or pcDNA3 using Lipofectamine 2000 (Gibco BRL, Carlsbad, USA). Following transfection, cells were maintained in RPMI 1640 medium containing 10% FBS and cultured for 48 h.

1.2 Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells with TRIzol reagent (Invitrogen Life Technologies, Inc., Carlsbad, CA) and quantified by UV absorbance spectroscopy. The reverse transcription reaction was performed using the Superscript First-Strand Synthesis System (Invitrogen Life Technologies, Inc., Carlsbad, CA) in a final volume of 20 ml containing 5 mg of total RNA, 200 ng of random hexamers, 1 × reverse transcription buffer, 2.5 mmol $MgCl_2$, 1 mmol deoxynucleotide triphosphate mixture, 10 mmol DTT, RNaseOUT recombinant ribonuclease inhibitor (Invitrogen), 50 U superscript reverse transcriptase, and diethylpyrocarbonate-treated water. After incubation at 42 ℃ for 50 min, the reverse transcription reaction was terminated by heating at 85 ℃ for 5 min. The newly synthesized cDNA was amplified by PCR. The reaction mixture contained 2 μl cDNA template, 1.5 mmol $MgCl_2$, 2.5 U Tag polymerase, and 0.5 mmol CXCL12 primer (5′- AGAGCCAACGTCAAGCATCT -3′; 5′- CGTCTTTGCCC TTTCATCTC-3′), PEA3 primer (5′-CAGCTCAGCTTCTTCCTAGGTC-3′; 5′-CCTCTCTGCTTATACCCAGCAC-3′). GAPDH (the human glyceraldehyde-3-phosphate dehydrogenase) primer (5′-GCCAAAAGGGTCATCATCTC-3′; 5′-GTA GAGGCAGGGATGATGTTC-3′) was used as an internal control. Amplification cycles were 94 ℃ for 3 min, then 33 cycles at 94 ℃ for 1 min, 58 ℃ for 1 min,

72 ℃ for 1.5 min, followed by 72 ℃ for 10 min. Aliquots of PCR product were electrophoresed on 1.5% agarose gels, and PCR fragments were visualized by ethidium bromide staining.

1.3 Real-time polymerase chain reaction (Real-time PCR)

Real-time quantitative PCR was performed using the fluorescence temperature cycler (Opticon, MJ Research) and the SYBR Green PCR Core Reagents kit according to the manufacturer's instructions (Takara, Dalian, China). The PCR products were detected by bound SYBR Green double-stranded DNA fluorescence, and the comparative threshold cycle ($2^{-\Delta\Delta cT}$) method was used to enable quantification of the mRNA of these genes. All samples were tested in triplicate. Target gene expression was compared to the housekeeping gene GAPDH. After PCR, a melting curve was obtained and analyzed.

1.4 Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were carried out according to the manufacturer's protocol (Active motif, Carlsbad, CA). Briefly, cells in 150-mm tissue culture dishes were fixed with 1% formaldehyde and incubated at 37 ℃ for 10 min. The cells were then washed twice with ice-cold phosphate-buffered saline (PBS), harvested, and re-suspended in ice-cold TNT lysis buffer [20 mmol Tris-HCl (pH 7.4), 200 mmol NaCl, 1% Triton X-100, 1 mmol PMSF (phenylmethanesul-fonylfluoride or phenylmethylsulfonyl fluoride) and 1% aprotinin]. The lysates were sonicated to shear the DNA to fragments of 200 – 600 bp, and subjected to immunoprecipitation with the following antibodies respectively, PEA3 or IgG (Santa Cruz Biotech-nology, Inc, Santa Cruz, CA). 3 mg antibodies were used for each immunoprecipitation. The antibody-protein complexes were collected by Protein G beads and washed three times with ChIP washing buffer (5% SDS, 1 mmol EDTA, 0.5% bovine serum albumin and 40 mmol $NaHPO_4$, pH 7.2). The immune complexes were eluted with 1% SDS and 1 mol $NaHCO_3$, and the cross-links were reversed by incubation in the presence of 200 mmol NaCl and RNase A at 65 ℃ for 4 h. The samples were then treated with proteinase K for 2 h, and then DNA was purified by mini-column, ethanol precipitation, and re-suspended in 100 ml of H_2O. The primer corresponding to the CXCL12 promoter region (−714 to −557) (sense: 5′-CACCATTGAGAGGTCGGAAG-3′; antisense: 5′- AATGAGACCCGTCTTTGCAG-

3′) was used for real time PCR to detect the presence of the CXCL12 promoter DNA. As for negative controls, we tested for the recruitment of PEA3 at exon 7 of the EGFR gene using the primers (sense: 5′-GCAATATCAGCCTTAGGTGCGGCTC-3′; antisense: 5′-CAT AGAAAGTGAACATTT AGGATGTG -3′).

1.5 Small interfering RNA (siRNA) preparation and transfection

Cells in the exponential phase of growth were seeded in 6-well plates at a concentration of 5×10^5 cells/well. After incubation for 24 h, the cells were transfected with siRNA specific for PEA3 (catalog number: 115237) (Ambion, Austin, TX, USA) and non-targeting siRNA at a final concentration of 100 nmol using oligofectamine and OPTI-MEMI reduced serum medium (Invitrogen Life Tech-nologies, Inc, Carlsbad, CA) according to the manufacturer's protocol. Silencing was examined 48 h after transfection by RT-PCR.

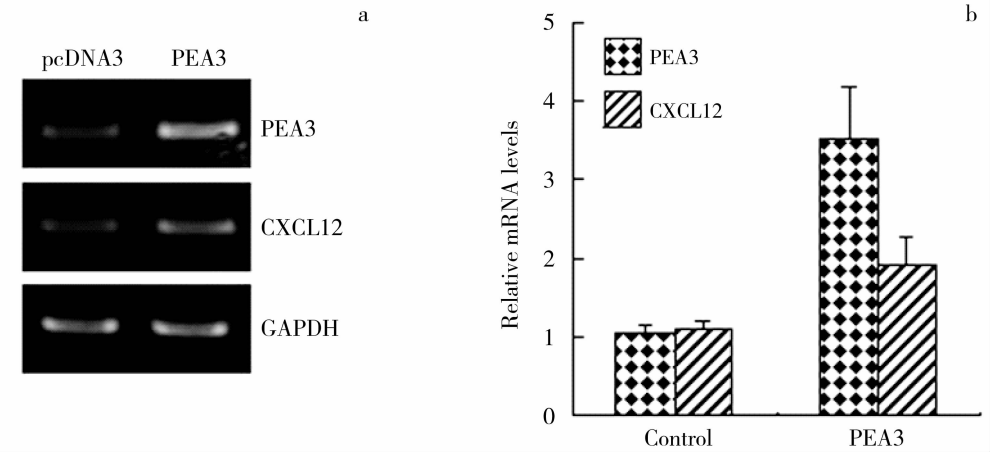
1.6 Luciferase reporter gene assay

MCF-7 cells were seeded in six-well plates at a density of $(1-2) \times 10^5$ cells/well and cultured for 24 h. Cells were then co-transfected with wild-type (pCMVLUC-SDF1010) or c-myb mutant (pCMVLUC-SDF1010mut) and CXCL12 reporter construct (0.5 mg/well), or cotransfected with 0.5 mg pcDNA3.0 or PEA3 expression vector together with 20 ng control Renilla luciferase reporter gene construct, pRL-TK (Promega, Madison, USA). The total amount of DNA per well was adjusted to 1.5 mg by the addition of sonicated salmon sperm DNA. Luciferase assays were performed as recommended by the manufacturer (Promega, Madison, WI) and normalized relatively to protein concentration determined by bicinchoninic acid protein assay (Pierce, Rockford, IL).

2 RESULTS

2.1 Overexpression of PEA3 induced CXCL12 mRNA level in MCF-7 cells

To identify the role of PEA3 in regulating CXCL12 transcription, PEA3 expression vector or pcDNA3 was transfected into MCF-7 cells and CXCL12 mRNA was detected. Fig 1(a,b) showed that as compared with MCF-7 cells transfected with pcDNA3, the level of CXCL12 mRNA in the cells transfected with PEA3 expression vector increased as determined by RT-PCR and Real-time PCR. In this experiment, exogenous PEA3 could induce CXCL12 mRNA, indicating PEA3 played a role in activating CXCL12 transcription.

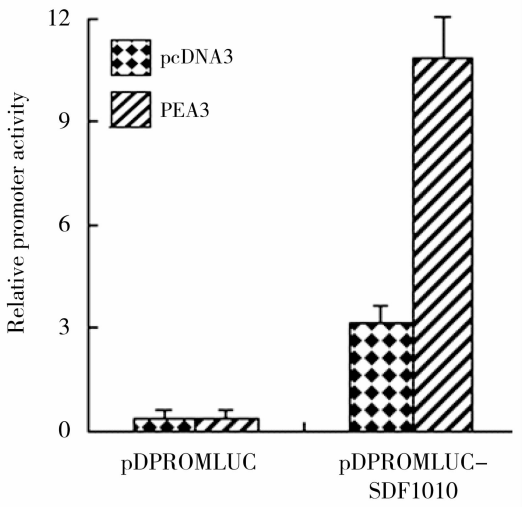


MCF-7 cells were plated in 6-well tissue culture plates, then transfected with 4 μg PEA3 expression vector or control vector, cultured for 48 h, mRNA expression levels of PEA3 and CXCL12 were detected by RT-PCR (a) and Real-time PCR (b).

Fig 1 Overexpression of PEA3 induced CXCL12 mRNA level in MCF-7 cells

2.2 PEA3 activated CXCL12 promoter activity in MCF-7 cells

To identify the role of PEA3 in regulating CXCL12 promoter transcription, we co-transfected the CXCL12 promoter/luciferase construct with PEA3 expression vector or pcDNA3 in MCF-7 cells and detected CXCL12 promoter activity. It was shown that the luciferase activity, which presents as "relative promoter activity" in Fig 2, was enhanced by PEA3 in MCF-7 cells; further indicating that PEA3 could activate CXCL12 promoter activity.

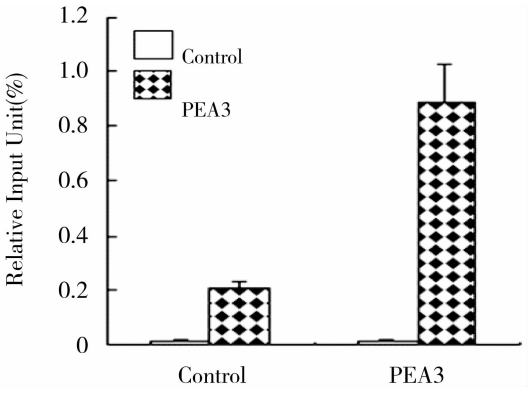


MCF-7 cells were plated in 6-well tissue culture plates, then co-transfected with 0.5 μg pCMVLUC or CXCL12 promoter construct (pCMVLUC-SDF1010) with 0.5 μg of PEA3 expression vector or pcDNA3.0 control vector for 48 h. Luciferase activity was detected as described in materials and methods.

Fig 2 PEA3 activated CXCL12 promoter activity in MCF-7 cells

2.3 PEA3 bound to the CXCL12 promoter in PEA3-overexpressed MCF-7 cells

To investigate if PEA3 could bind to the CXCL12 promoter in the cells transfected with PEA3 expression vector, we performed ChIP experiment. The result showed that PEA3 could bind to the CXCL12 promoter in MCF-7 cells transfected with PEA3 expression vector (Fig 3). In this experiment, PEA3 could bind to the CXCL12 promoter in PEA3-overexpressed MCF-7 cells, indicating PEA3 activated CXCL12 transcription by binding directly to the CXCL12 promoter.

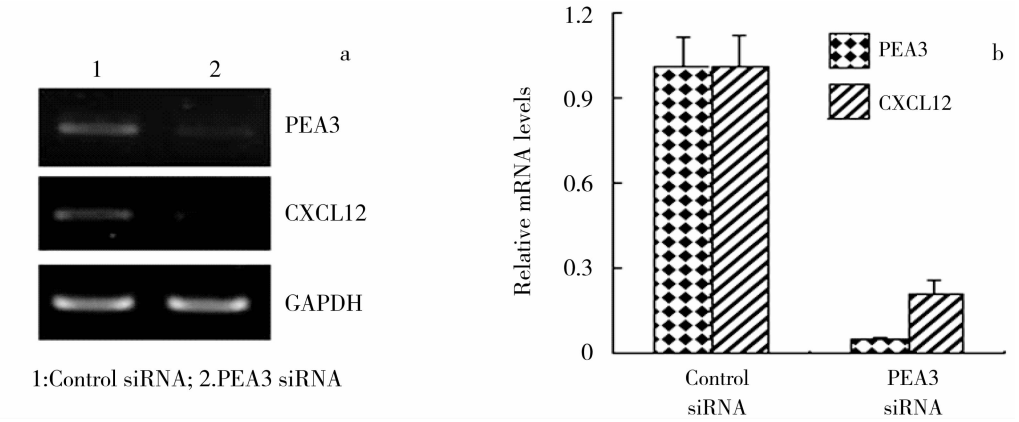


MCF-7 cells were plated in 6-well tissue culture plates, then transfected with 4 μg PEA3 expression vector, cultured for 48 h. Nucleic extracts were prepared from MCF-7 cells with or without PEA3 transfection. ChIP assays were performed as described in materials and methods. The primers corresponding to the CXCL12 promoter region (−714 to −557) upstream of the transcriptional start site were used for PCR to detect the presence of the CXCL12 promoter DNA.

Fig 3 PEA3 bound to the CXCL12 promoter after overexpression of PEA3 in MCF-7 cells

2.4 PEA3 siRNA inhibited CXCL12 transcription in MCF-7 cells

To further identify the role of PEA3 in regulating CXCL12 transcription, we knocked down the expression of PEA3 with a gene-specific siRNA and measured PEA3 mRNA by RT-PCR and Real-time PCR. As shown in Fig 4(a, b), PEA3 siRNA inhibited PEA3 mRNA significantly in MCF-7 cells after transfection with PEA3 siRNA for 48 h. And it also inhibited CXCL12 mRNA in the MCF-7 cells. This experiment indicated that PEA3 siRNA could knock down PEA3 expression efficiently and decrease CXCL12 mRNA expression significantly.

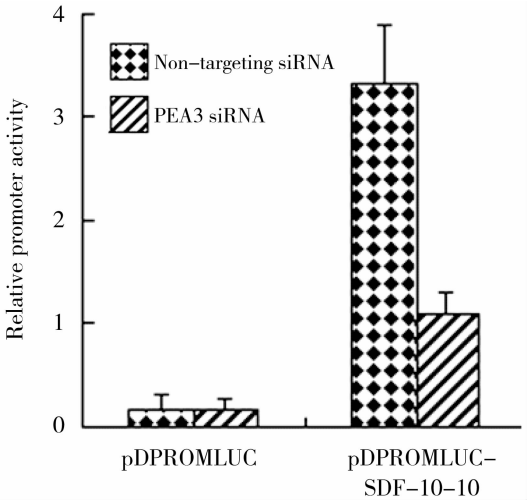


MCF-7 cells were treated with 100 nmol PEA3 siRNA or non-targeting siRNA for 48 h, RT-PCR (a) and Real-time PCR (b) analyses were performed as described in materials and methods. GAPDH was used as an internal control.

Fig 4 PEA3 siRNA inhibited PEA3 and CXCL12 mRNA in MCF-7 cells

2.5 PEA3 siRNA repressed CXCL12 promoter activity in MCF-7 cells

To determine if the decrease of PEA3 would reduce CXCL12 gene transcription, we knocked down the expression of PEA3 and measured CXCL12 promoter activity. As shown in Fig 5, PEA3 siRNA attenuated the CXCL12 promoter activity in MCF-7 cells after transfection with PEA3 siRNA for 48 h. This experiment indicated that when endogenous PEA3 was knocked down by siRNA, the promoter activity of endogenous CXCL12 decreased.

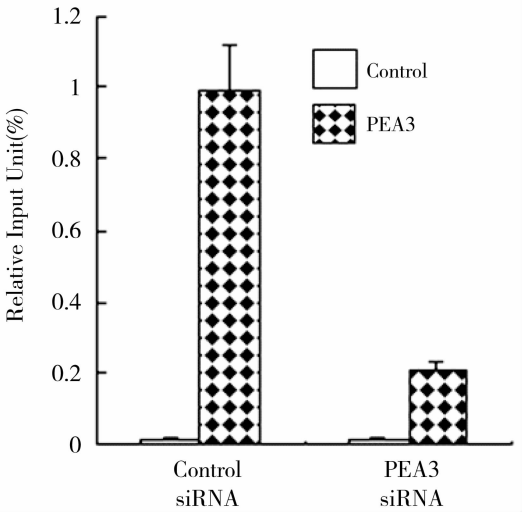


MCF-7 cells were plated in 6-well tissue culture plates, then pCMVLUC or CXCL12 promoter construct (pCMVLUC-SDF1010) was co-transfected with PEA3 siRNA or non-targeting RNA into cells, cultured for 48 h. Luciferase activity was detected as described in materials and methods.

Fig 5 PEA3 siRNA repressed CXCL12 promoter activity in MCF-7 cells

2.6 PEA3 siRNA attenuated the binding of PEA3 to the CXCL12 promoter

To determine if the decrease of PEA3 would influence the binding of PEA3 onto the CXCL12 promoter, we knocked down the expression of PEA3 and measured the binding status of PEA3 on the CXCL12 promoter. As demonstrated in Fig 6, PEA3 siRNA attenuated the binding of PEA3 to the CXCL12 promoter in MCF-7 cells after transfection with PEA3 siRNA for 48 h. This experiment showed that when endogenous PEA3 was knocked down by siRNA, the binding of PEA3 to the CXCL12 promoter decreased, also indicating that PEA3 regulated CXCL12 transcription by binding directly to the CXCL12 promoter.



MCF-7 cells were treated with 100 nmol PEA3 siRNA or non-targeting siRNA (Contro siRNA) for 48 h and ChIP assay was performed.

Fig 6 PEA3 siRNA attenuated the binding of PEA3 to the CXCL12 promoter

3 DISCUSSION

The PEA3 transcription factor belongs to the Ets family[14-16]. PEA3 is involved in both normal development and oncogenesis. PEA3 is overexpressed in triple-negative and other breast cancer subtypes[17-18]. Both VEGF (vascular endothelial growth factor) and HER2/neu are genes related to breast cancer. Reporter assays demonstrate that VEGF and HER2/neu can be activated via PEA3 in breast cancer cell lines[19-20]. Recent studies showed that ERβ and PEA3 co-activate IL-8 expression and promote the invasion of breast cancer cells. And also

PEA3 was involved in the Wnt1-induced tumorigenesis. All of the above provides evidence for a protumorigenic role of PEA3 factors in breast neoplasia, and supports targeting the PEA3 transcription factor family in breast cancer[21-23].

Manual knockout of PEA3 reduced tumor growth in breast cancer cell lines. PEA3 has a close correlation to cancer characteristic. But up to now there are few reports about the interaction between PEA3 and CXCL12 transcription in breast cancer.

In our studies, overexpression of PEA3 could increase the CXCL12 mRNA level in MCF-7 cells. In order to analyse the putative effects of PEA3 on CXCL12 transcription, we performed luciferase assay, and our results demonstrated that PEA3 activated CXCL12 promoter activity. ChIP assay demonstrated that PEA3 could bind to the CXCL12 promoter in the PEA3-overexpressed cells. Bioinformatic analysis of the 5'-flanking region of the human CXCL12 gene showed that there existed a PEA3 binding site (TTTCCT) in the CXCL12 promoter region from −631 to −626. It has been reported that PEA3 could elevate PEG-3 promoter activity by binding to the PEA3 binding site (TTTCCT) in the PEG-3 promoter[24-25].

A functional interaction between PEA3 and CXCL12 promoter was strengthened by PEA3 siRNA. We further found that PEA3 siRNA attenuated the CXCL12 promoter activity in MCF-7 cells. At the same time, PEA3 siRNA attenuated the binding of PEA3 to the CXCL12 promoter. These results further indicated that PEA3 could influence the CXCL12 promoter activity by binding to the CXCL12 promoter.

We conclude that PEA3 plays an important role in inducing the CXCL12 promoter activity by directly binding to the CXCL12 promoter. These investigations are important and offer potential for defining the angiogenic mechanism regulated by CXCL12 and PEA3. With this information, it will be possible to demarcate potential targets and define appropriate reagents, such as antisense or small molecule antagonists, for inhibiting or preventing breast cancer development and progression.

References

[1] Bleul CC, Fuhlbrigge RC, Casasnovas JM, et al. A highly efficacious lymphocyte chemoattractant, stromal cell-derived

factor 1 (SDF-1) [J]. J Exp Med, 1996, 184(3): 1101-1109.

[2] Balabanian K, Lagane B, Infantino S, et al. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes [J]. J Biol Chem, 2005, 280(42): 35 760-35 766.

[3] Sun X, Cheng G, Hao M, et al. CXCL12 / CXCR4 / CXCR7 chemokine axis and cancer progression [J]. Cancer Metastasis Rev, 2010, 29(4): 709-722.

[4] Teicher BA, Fricker SP. CXCL12 (SDF-1)/CXCR4 pathway in cancer [J]. Clin Cancer Res, 2010, 16(11): 2927-2931.

[5] Vlahakis SR, Villasis-Keever A, Gomez T, et al. G protein-coupled chemokine receptors induce both survival and apoptotic signaling pathways [J]. J Immunol, 2002, 169(10): 5546-5554.

[6] Bachelder RE, Wendt MA, Mercurio AM. Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4 [J]. Cancer Res, 2002, 62(24): 7203-7206.

[7] Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis [J]. Nature, 2001, 410(6824): 50-56.

[8] Hassan S, Baccarelli A, Salvucci O, et al. Plasma stromal cell-derived factor-1: host derived marker predictive of distant metastasis in breast cancer [J]. Clin Cancer Res, 2008, 14(2): 446-454.

[9] Zhou W, Jiang Z, Song X, et al. Promoter hypermethylation-mediated down-regulation of CXCL12 in human astrocytoma [J]. J Neurosci Res, 2008, 86(13): 3002-3010.

[10] Sowin′ska A, Jagodzinski PP. RNA interference-mediated knockdown of DNMT1 and DNMT3B induces CXCL12 expression in MCF-7 breast cancer and AsPC1 pancreatic carcinoma cell lines [J]. Cancer Lett, 2007, 255(1): 153-159.

[11] Wendt MK, Johanesen PA, Kang-Decker N, et al. Silencing of epithelial CXCL12 expression by DNA hypermethylation promotes colonic carcinoma metastasis [J]. Oncogene, 2006, 36(36): 4986-4997.

[12] Pietkiewicz PP, Lutkowska A, Lianeri M, et al. Tamoxifen epigenetically modulates CXCL12 expression in MCF-7 breast [J]. Biomed Pharmacother, 2010, 64(1): 54-57.

[13] Chen L, Xu SG, Zeng XH, et al. c-myb activates CXCL12 transcription in T47D and MCF7 breast cancer cells cancer cells [J]. Acta Biochim Biophys Sin, 2010, 42(1): 1-7.

[14] Xin JH, Cowie A, Lachance P, et al. Molecular cloning and characterization of PEA3, a new member of the Ets oncogene family that is differentially expressed in mouse embryonic cells [J]. Genes Dev, 1992, 6(3): 481-496.

[15] Monte D, Coutte L, Baert JL, et al. Molecular characterization of the Ets-related human transcription factor ER81 [J]. Oncogene, 1995, 11(4): 771-779.

[16] Monte D, Baert JL, Defossez PA, et al. Molecular cloning and characterization of human ERM, a new member of the Ets family closely related to mouse PEA3 and ER81 transcription factors. [J]. Oncogene, 1994, 9(5): 1397-1406.

[17] Clementz AG, Rogowski AS, Pandya K, et al. Notch-1 and Notch-4 are novel gene targets of pea3 in breast cancer: novel therapeutic implications[J]. Breast Cancer Res, 2011, 13(3): R63.

[18] Shepherd TG, Kockeritz L, Szrajber MR, et al. The pea3 subfamily ets genes are required for HER2/Neu-mediated mammary oncogenesis [J]. Curr Biol, 2001, 11(22): 1739-1748.

[19] Hua D, Chen B, Bai M, et al. PEA3 activates VEGF transcription in T47D and SKBR3 breast cancer cells [J]. Acta Biochim Biophys Sin, 2009, 41(1): 63-68.

[20] Ni J, Kalff-Suske M, Gentz R, et al. All human genes of the uteroglobin family are localized on chromosome 11q12.2 and form a dense cluster [J]. Ann NY Acad Sci, 2000, 12(923): 25-42.

[21] Chen Y, Chen L, Li JY, et al. ERβ and PEA3 co-activate IL-8 expression and promote the invasion of breast cancer cells [J]. Cancer Biol Ther, 2011, 11(5): 497-511.

[22] Baker R, Kent CV, Silbermann RA, et al. Pea3 transcription factors and wnt1-induced mouse mammary neoplasia [J]. PLoS One, 2010, 5(1): e8854.

[23] Qin L, Liu Z, Chen H, et al. The steroid receptor coactivator-1 regulates twist expression and promotes breast cancer metastasis [J]. Cancer Res, 2009,69(9):3819-3827.

[24] Su Z, Shi Y, Friedman R, et al. PEA3 sites within the progression elevated gene-3 (PEG-3) promoter and mitogen-activated protein kinase contribute to differential PEG-3 expression in Ha-ras and v-raf oncogene transformed rat embryo cells [J]. Nucleic Acids Res,2001,29(8):1661-1671.

[25] Su ZZ, Shi YJ, Fisher PB. Cooperation between AP1 and PEA3 sites within the progression elevated gene-3 (PEG-3) promoter regulate basal and differential expression of PEG-3 during progression of the oncogenic phenotype in transformed rat embryo cells [J]. Oncogene,2000,19(30):3411-3421.

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